include, but are not limited to, use of mismatches or base analogs in the hairpin duplex at one, a few or all positions, similar disruptions or modifications in the duplex between the primer and the 3' arm of the S-60, chemical or other modifications to one or both ends of the primer sequence, or attachment of moieties to, or other modifications of the 5' arm of the structure. In all of the analyses using the S-60 or a similar hairpin described herein, activity with and without a primer may be compared using the same hairpin structure.

The assembly of these test reactions, including appropriate amounts of hairpin, primer and candidate nuclease are described in Example 2. As cited therein, the presence of cleavage products is indicated by the presence of molecules which migrate at a lower molecular weight than does the uncleaved test structure. When the reversal of charge of a label is used the products will carry a different net charge than the uncleaved material. Any of these cleavage products indicate that the candidate nuclease has the desired structure-specific nuclease activity. By "desired structure-specific nuclease activity" it is meant only that the candidate nuclease cleaves one or more test molecules. It is not necessary that the candidate nuclease cleave at any particular rate or site of cleavage to be considered successful cleavage.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the disclosure which follows, the following abbreviations apply:°C (degrees Centigrade); g (gravitational field); vol (volume); w/v (weight to volume); v/v (volume to volume); BSA (bovine serum albumin); CTAB (cetyltrimethylammonium bromide); HPLC (high pressure liquid chromatography); DNA (deoxyribonucleic acid); p (plasmid); μl (microliters); ml (milliliters); μg (micrograms); pmoles (picomoles); mg (milligrams); M (molar); mM (milliMolar); μM (microMolar); nm (nanometers); kdal (kilodaltons); OD (optical density); EDTA (ethylene diamine tetra-acetic acid);

FITC (fluorescein isothiocyanate); SDS (sodium dodecyl sulfate); $NaPO_4$ (sodium phosphate); Tris (tris(hydroxymethyl)-aminomethane); PMSF (phenylmethylsulfonylfluoride); TBE (Tris-Borate-EDTA, *i.e.*, Tris buffer titrated with boric acid rather than HCl and containing EDTA) ; PBS (phosphate buffered saline); PPBS (phosphate buffered saline containing 1 mM PMSF); PAGE (polyacrylamide gel electrophoresis); Tween (polyoxyethylene-sorbitan); Dynal (Dynal A.S., Oslo, Norway); Epicentre (Epicentre Technologies, Madison, WI); MJ Research (MJ Research, Watertown,MA); National Biosciences (Plymouth, MN); New England Biolabs (Beverly, MA); Novagen (Novagen, Inc., Madison, WI); Perkin Elmer (Norwalk, CT); Promega Corp. (Madison, WI); Stratagene (Stratagene Cloning Systems, La Jolla, CA); USB (U.S. Biochemical, Cleveland, OH).

EXAMPLE 1

Characteristics Of Native Thermostable DNA Polymerases

A. 5' Nuclease Activity Of DNAP*Taq*

During the polymerase chain reaction (PCR) [Saiki *et al.*, *Science* 239:487 (1988); Mullis and Faloona, *Methods in Enzymology* 155:335 (1987)], DNAP*Taq* is able to amplify many, but not all, DNA sequences. One sequence that cannot be amplified using DNAP*Taq* is shown in Figure 6 (Hairpin structure is SEQ ID NO:15, PRIMERS are SEQ ID NOS:16-17.) This DNA sequence has the distinguishing characteristic of being able to fold on itself to form a hairpin with two single-stranded arms, which correspond to the primers used in PCR.

To test whether this failure to amplify is due to the 5' nuclease activity of the enzyme, we compared the abilities of DNAP*Taq* and DNAPStf to amplify this DNA sequence during 30 cycles of PCR. Synthetic oligonucleotides were obtained from The Biotechnology Center at the University of Wisconsin-Madison. The DNAP*Taq* and DNAPStf were from Perkin Elmer (*i.e.*, Amplitaq™ DNA polymerase and the Stoffel fragment of Amplitaq™ DNA polymerase). The substrate DNA comprised the

hairpin structure shown in Figure 6 cloned in a double-stranded form into pUC19. The primers used in the amplification are listed as SEQ ID NOS:16-17. Primer SEQ ID NO:17 is shown annealed to the 3' arm of the hairpin structure in Fig. 6. Primer SEQ ID NO:16 is shown as the first 20 nucleotides in bold on the 5' arm of the hairpin in Fig. 6.

Polymerase chain reactions comprised 1 ng of supercoiled plasmid target DNA, 5 pmoles of each primer, 40 μM each dNTP, and 2.5 units of DNAP*Taq* or DNAPStf, in a 50 μl solution of 10 mM Tris•Cl pH 8.3. The DNAP*Taq* reactions included 50 mM KCl and 1.5 mM $MgCl_2$. The temperature profile was 95°C for 30 sec., 55°C for 1 min. and 72°C for 1 min., through 30 cycles. Ten percent of each reaction was analyzed by gel electrophoresis through 6% polyacrylamide (cross-linked 29:1) in a buffer of 45 mM Tris•Borate, pH 8.3, 1.4 mM EDTA.

The results are shown in Figure 7. The expected product was made by DNAPStf (indicated simply as "S") but not by DNAP*Taq* (indicated as "T"). We conclude that the 5' nuclease activity of DNAP*Taq* is responsible for the lack of amplification of this DNA sequence.

To test whether the 5' unpaired nucleotides in the substrate region of this structured DNA are removed by DNAP*Taq*, the fate of the end-labeled 5' arm during four cycles of PCR was compared using the same two polymerases (Figure. 8). The hairpin templates, such as the one described in Figure 6, were made using DNAPStf and a ^{32}P-5'-end-labeled primer. The 5'-end of the DNA was released as a few large fragments by DNAP*Taq* but not by DNAPStf. The sizes of these fragments (based on their mobilities) show that they contain most or all of the unpaired 5' arm of the DNA. Thus, cleavage occurs at or near the base of the bifurcated duplex. These released fragments terminate with 3' OH groups, as evidenced by direct sequence analysis, and the abilities of the fragments to be extended by terminal deoxynucleotidyl transferase.

Figures 9-11 show the results of experiments designed to characterize the cleavage reaction catalyzed by DNAP*Taq*. Unless otherwise specified, the cleavage